Method Development and Validation of Lisinopril Dihydrate and Hydrochlorothiazide in Bulk and Tablet Dosage Form by RP-HPLC

Girish C[1], Paul Richards M[2], Harini Kumari G[3], Venu Priya R[4]

Bharath Rathna Kumar P[5]*

[1]Department of Pharmacology, SVU College of Pharmaceutical Sciences, SV University, Tirupati, Andhra Pradesh, India.
[2]Department of Pharmaceutical Analysis, Aditya Bangalore Institute of Pharmacy Education and Research, Yelhanka, Bangalore, India.
[3]Department of Pharmaceutics, East point College of Pharmacy, Bidarahalli, Bangalore, Karnataka, India.
[4]Department of Pharmaceutics, Anwarul Uloom College of Pharmacy, Mallepally, Hyderabad, India.
*[5]Department of Pharmaceutical Chemistry, Anwarul Uloom College of Pharmacy, Mallepally, Hyderabad, India. *Corresponding author*

ABSTRACT

A simple, sensitive and precise high performance liquid chromatographic method for the analysis of Lisinopril Dihydrate and Hydochlorothiazide has been developed and validated for the determination of compounds in commercial pharmaceutical products. The compounds were well separated on Phenominex C18 (250 mm× 4.6 mm × 5 µ) reversed-phase column by use of a mobile phase consisting of phosphate buffer (potassium dihydrogen orthophosphate) and acetonitrile of pH adjusted to 3.5 with ortho phosphoric acid in 65:35 v/v ratio, at a flow rate of 1.0 ml/min with detection wave length at 233 nm. The retention times of Lisinopril Dihydrate and Hydrochlorothiazide are 2.347 min and 3.944 min respectively. The linearity ranges were 2.5-15 µg. The recovery amount was more than 99%. The high recovery and low relative standard deviation conforms the suitability of the method for the determination of Lisinopril Dihydrate and Hydrochlorothiazide in tablet dosage form.

KEY WORDS: Lisinopril dihydrate, Hydrochlorothiazide, RP-HPLC, Method development, Method validation

INTRODUCTION

Lisinopril[1] is a drug of the angiotensin converting enzyme (ACE) inhibitor class primarily used in treatment of hypertension, congestive heart failure, and heart attacks and also in preventing renal and retinal complications of diabetes. Its indications, contraindications and side effects are as those for all ACE inhibitors. It is designated chemically *N*2-[(1*S*)-1-

carboxy-3-phenylpropyl]-L-Lysol-L-proline and its empirical formula is $C_{21}H_{31}N_3O_5$ and its structure is shown in Fig.1

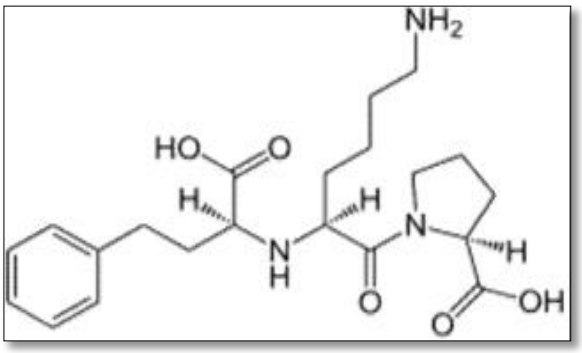

Fig.1 Structure of Lisinopril

Hydrochlorothiazide[2] is white crystalline compound, soluble in water, but freely soluble in sodium hydroxide solution with molecular weight 297.74. Its IUPAC name is 6-chloro-1, 1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide. It is a first line diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus the cardiac output, is believed to lower peripheral vascular resistance. Its empirical formula is $C_7H_8ClN_3O_4S_2$ and its structure is shown in Fig.2.

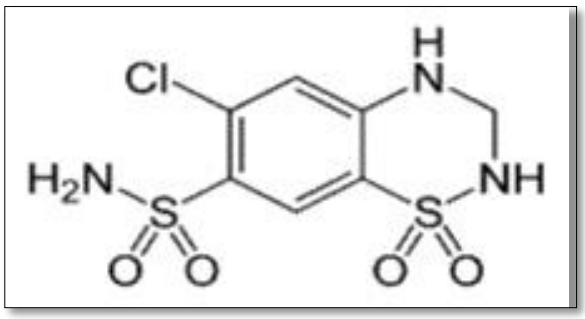

Fig.2 Structure of Hydrochlorothiazide

The present work is focused on HPLC method development and validation of drug by RP-HPLC. A thorough literature review was conducted it was found that many methods were used for identification of this active pharmaceutical ingredient either individually or in combination with other drug by UV spectrophotometry[3-10], HPLC[11-14], HPTLC[15] and there are very few reported methods for determination of Lisinopril Dihydrate and Hydrochlorothiazide

by simultaneous RP-HPLC determination. Hence the present work is to develop and validate method for the simultaneous estimation of Lisinopril Dihydrate and Hydrochlorothiazide .

EXPERIMENTAL

MATERIALS AND METHODS

The pharmaceutical drug samples Lisinopril Dihydrate and Hydrochlorothiazide were as a gift from Bio-leo Labs, Hyderabad. All the chemicals used of HPLC grade such as Ammonium dihydrogen Phosphate Buffer was obtained from Rankem (RFCL Limited) Manufacturers and Acetonitrile was purchased from Thermo Fischer Scientific India, Pvt. Ltd, used as a mobile phase. Water used in the buffer preparation was freshly prepared from Milli-Q, NA.

Chromatographic Conditions

Waters e-2695 empower-2 HPLC is used for the method development and validation of Lisinopril Dihydrate and Hydrochlorothiazide in API and pharmaceutical preparations. UV PROBE- 2450 UV/VIS spectrophotometer is used for the detection of Lisinopril Dihydrate and Hydrochlorothiazide in API and pharmaceutical preparations.

METHOD DEVELOPMENT

Chromatographic Method Selection

The drug Lisinopril Dihydrate and hydrochlorothzide was polar in nature. For the method development of the drug reverse phase or ion exchange or ion pair chromatography methods are suitable. The reverse phase HPLC method was selected for the initial separation owing to its simplicity, suitability, robustness, ruggedness and its wider usage.

Wavelength Selection

Individual drug components such as Lisinopril Dihydrate were detected at 211 nm and Hydrochlorothiazide was detected at 270 nm. Mixed standard overlay spectrum of Lisinopril Dihydrate and Hydrochlorothiazide drug showed an isobestic point at 233 nm hence this wavelength was selected as the detection wavelength for HPLC.

Mobile Phase Selection

The drug Lisinopril Dihydrate and Hydrochlorothiazide was completely soluble in water and methanol. Combination of Phosphate buffer (Potassium di hydrogen ortho phosphate) and Acetonitrile in the ratio of 65:35 v/v (pH- 3.5 maintained with Ortho phosphoric acid) was suitable for the method development and validation of the drug.

Stationary Phase (Column) Selection

Different columns usually have different selectivity for drug components. The column Phenominex C18 (250mm×4.6mm×5µ) was more selective for the drug Lisinopril Dihydrate and Hydrochlorothiazide in the method development and validation.

Preparation of analytical Solutions

Preparation of standard Lisinopril Dihydrate Solution

An accurately weighed quantity of 1 mg of standard Lisinopril Dihydrate was dissolved in 10ml of mobile phase (Phosphate buffer : Acetonitrile in the ratio of 65:35 v/v) to get the concentration of 100 µg/ml and sonicated for 5 min for proper dissolution.

Preparation of standard Hydrochlorothiazide Solution

An accurately weighed quantity of 1mg of standard Hydrochlorothiazide was dissolved in 10ml of mobile phase (Phosphate buffer : Acetonitrile in the ratio of 65:35 v/v) to get the concentration of 100 µg/ml and sonicated for 5 min for proper dissolution.

Preparation of Mixed standard solution

Mixed standard solution was prepared by adding 5 ml of standard solution of Lisinopril Dihydrate and 5 ml standard solution of Hydrochlorothiazide in 10ml volumetric flask and the resultant solution was mixed properly.

Preparation of Sample Solution (marketed formulation)

Twenty tablets were accurately weighed and the average weight was 192.5 mg and the sample weight observed is 196.5 mg which contained equivalent weight to 5mg of Lisinopril Dihydrate and 12.5 mg of Hydrochlorothiazide. 1 mg of sample was accurately weighed and dissolved in 10 ml of mobile phase to get 100 µg/ml concentration and the sample solution sonicated for 5 min for proper dissolution. Sample and standard solutions were injected into to the chromatographic system separately. Equal volume of blank (mobile phase) solution is injected into the system. The chromatograms were recorded for sample and standard injections eliminating the peak area of blank.

Potassium Di Hydrogen Ortho Phosphate (0.01N)

An accurately weighed quantity of 680.45 mg of Potassium dihydrogen ortho phosphate was dissolved in 500 ml of HPLC water and sonicated for 10min for proper dissolution and adjusted to the pH 3.5 with Orthophosporic acid then filtered.

Hydrochloric Acid (0.1N)

An accurately measured volume of 4.25 ml of Hydrochloric acid was dissolved in 30ml of water and final volume made to 500 ml with distilled water.

Sodium Hydroxide (0.1N)

An accurately weighed quantity of 2 g of Sodium hydroxide was dissolved in 30 ml of water and sonicated for 10 min for proper dissolution and the final volume made to 500 ml with distilled water.

METHOD VALIDATION

The method development of Lisinopril Dihydrate and Hydrochlorothiazide should be validated according to ICH guidelines including parameters such as accuracy, precision, linearity, range, specificity, system suitability, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness.

Accuracy

Accuracy test is intended to determine the closeness of the value found which is accepted either as a conventional true value or as an accepted reference value. Accuracy was determined in terms of percentage recovery. 50, 100, 150 µ/ml of sample and standard solutions are prepared injected into the system. The percentage recoveries of Lisinopril Dihydrate and Hydrochlorothiazide were calculated.

Precision

Precision was determined both in terms of repeatability and intermediate precision. 20 tablets were accurately weighed and the average weight was 192.5 mg and the sample weight observed is 196.5 mg which contained an equivalent weight to 5 mg of Lisinopril Dihydrate and 12.5 mg of Hydrochlorothiazide. 1mg of sample was accurately weighed and dissolved in 10 ml of mobile phase to get 100 µg/ml concentration and sonicated for 5 min for proper dissolution. The solution injected thrice into the chromatographic system.

Linearity

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. 20 tablets were accurately weighed and the average weight was 192.5 mg and the sample weight observed is 196.5 mg which contained a equivalent weight to 5 mg of lisinoprildihydrate and 12.5 mg of Hydrochlorothiazide. 1mg of sample was accurately weighed and dissolved in 10ml of mobile phase and sonicated for 5min

for proper dissolution. The resultant solution was further diluted to get 25, 50, 75, 100, 125 and 150 µg/ml concentrations. These solutions injected into chromatographic system.

Range

The range of an analytical procedure is the interval between the upper and lower concentrations of analyte in the sample. The range for the Lisinopril Dihydrate and Hydrochlorothiazide was 100-150 µg/ml.

Specificity

Specificity of the method was found to determine the non-interference of the blank, internal standard and mobile phase. The mobile phase and sample solutions were injected into chromatographic system separately and obtained chromatograms were shown in fig.3. The method showed excellent specificity with Lisinopril Dihydrate and Hydrochlorothiazide eluted at 2.347 min and 3.944 min retention times. No interference was observed with mobile phase.

System suitability

According to ICH guidelines System suitability is defined as the checking of a system, before or during analysis of unknowns, to ensure system performance. Mixed standard solutions were prepared by adding 1ml of the standard Lisinopril Dihydrate solution to 1ml of standard Hydrochlorothiazide solution in 10ml volumetric flask the volume made with mobile phase to get 100 µg/ml concentration and sonicated for 5 min for proper dissolution. Six replicate injections of standard solution were injected in to the chromatographic system.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. 20 tablets were accurately weighed and the average weight was 192.5 mg and the sample weight observed is 196.5 mg which contained a equivalent weight to 5 mg of Lisinopril Dihydrate and 12.5 mg of Hydrochlorothiazide. 1mg of sample was accurately weighed and dissolved in 10ml of mobile phase and sonicated for 5min for proper dissolution. The resultant solution was further diluted to get a concentration of 80 µg/ml. and the solution injected into the chromatographic system at the flow rate of 0.8 ml/min and 1.2 ml/min respectively.

Ruggedness

According to USP, ruggedness is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. 20 tablets were accurately weighed and the average weight was 192.5 mg and the sample weight observed is 196.5 mg which contained an equivalent weight to 5 mg of Lisinopril Dihydrate and 12.5 mg of Hydrochlorothiazide. 1mg of sample was accurately weighed and dissolved in 10ml of mobile phase and sonicated for 5 min for proper dissolution. The resultant solution was further diluted to get a concentration of 60 µg/ml. The solution injected into chromatographic system.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Lisinopril Dihydrate and Hydochlorothiazide. The method developed was proceeded with wavelength selection. The optimized wavelength was 233nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions: The compounds were well separated on Phenominex C18 (250 mm× 4.6 mm × 5 µ) reversed-phase column by use of a mobile phase consisting of phosphate buffer (potassium dihydrogen orthophosphate) and acetonitrile of pH adjusted to 3.5 with ortho phosphoric acid in 65:35 v/v ratio, at a flow rate of 1.0 ml/min with detection wave length at 233 nm. The retention times of Lisinopril Dihydrate and Hydrochlorothiazide are 2.347 min and 3.944 min respectively. The linearity ranges were 2.5-15 µg. The recovery amount was more than 99%.

Table1.Peak area and RT of Lisinopril Dihydrate and Hydrochlorothiazide

Peak Name	RT (min)	Area	Height	%Area	Resolution	USP Plate count	Symmetry factor
Lisinopril dihydrate	2.347	787513	96059	16.99		2878	1.23
Hydrochlorothiazide	3.944	3848394	388335	83.01	6.85	3819	1.28

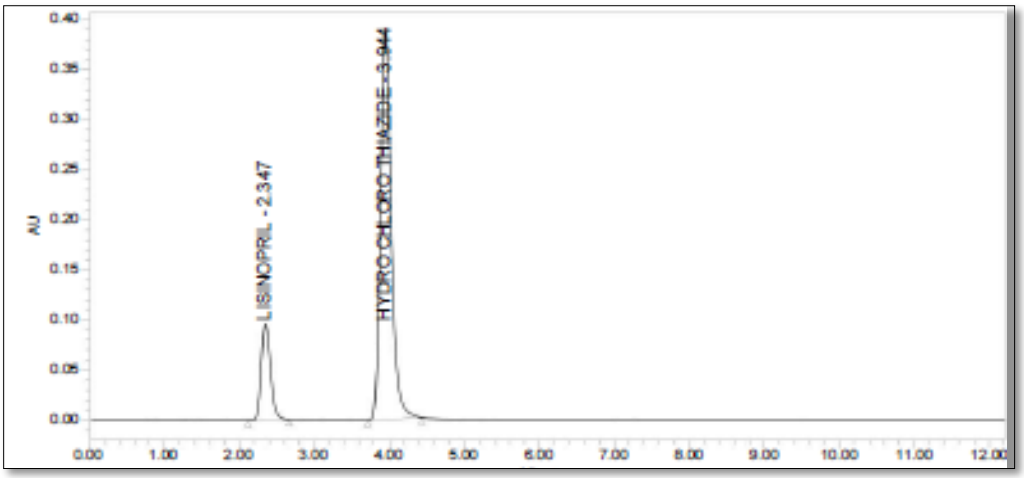

Fig.3. RP-HPLC Chromatograph of Lisinopril Dihydrate and Hydrochlorothiazide.

METHOD VALIDATION

Accuracy

Average recoveries of Lisinopril Dihydrate and Hydrochlorothiazide are 99.48% and 99.57 respectively. The percentage recoveries of drug components are within the limits 95-101%. So the method is accurate. The recovery data is shown in Table.2.

Table.2. Accuracy of Lisinopril Dihydrate and Hydrochlorothiazide

S.No	Drug	Conc.	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	Lisinopril dihydrate	50%	390309	50	49.97	99.4	99.48
		100%	777818.66	100	99.5	99.64	
		150%	2025922	150	149.45	99.64	
2	Hydro chlorothiazide	50%	2168905.1	50	49.82	99.64	99.57
		100%	4343144.3	100	99.16	99.76	
		150%	6485103	150	148.96	99.31	

Precision

Precision data represents both method precision and system precisions, summarized in table 3 and 4. The %RSD values for both method precision and system precision were less than 2.0%, which indicate that the proposed method is precise.

Table no.3: Method Precision

S.No	No. of injections	Area of lisinopril dihydrate	Area of hydrochlorothzide
1	Injection-1	802365	4302548
2	Injection-2	790564	4295481
3	Injection-3	801453	4365425
4	Injection-4	799458	4302187
5	Injection-5	794845	4265685
6	Injection-6	790125	4301253
Average		796468.3333	4305431.5
Standard Deviation		5409.065119	32601.1556
%RSD		0.68	0.76

Table 4: System Precision

S.No	No. of injections	Area of lisinopril dihydrate	Area of hydrochlorothzide
1	Injection-1	793350	4312014
2	Injection-2	803583	4362876
3	Injection-3	802885	4307544
4	Injection-4	791176	4351177
5	Injection-5	789476	4292648
6	Injection-6	788660	4337454
Average		794846.6667	4327285.5
Standard Deviation		6697.91907	27435.6986
%RSD		0.84	0.63

Linearity

The response was linear over concentrations of 0.0025 µg/ml, 0.005 µg/ml, 0.0075 µg/ml, 0.01µg/ml, 0.0125 µg/ml and 0.015 µg/ml for Lisinopril Dihydrate and Hydrochlorothiazide. The correlation co-efficient for both drugs were found to be 0.999. So the method is linear. The linearity levels of both drugs represented in table 5.

Table 5: The linearity levels of Lisinopril dihydrate and Hydrochlorothiazide

S.No	Linearity level	Lisinopril dihydrate		Hydrochlorothiazide	
		Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	Level-1	0.0025	203041	0.0025	1163326
2	Level-2	0.005	406150	0.005	2291175
3	Level-3	0.0075	596855	0.0075	3327756
4	Level-4	0.01	789924	0.01	4338563
5	Level-5	0.0125	1010298	0.0125	5351204
6	Level-6	0.015	1233348	0.015	63346699
Correlation co-efficient		0.9994		0.9995	

Robustness

Small deliberate variations in experimental parameters such as flow rate and buffer not significantly affect the recoveries and peak areas, peak area and retention time of drugs that indicates that the proposed method is robust. The robustness values of Lisinopril dihydrate and Hydrochlorothiazide are shown in Table 6.

Table 6: The robustness values of Lisinopril dihydrate and Hydrochlorothiazide

Parameter		Lisinopril dihydrate		Hydrochlorothiazide	
Changes in flow rate	ml/min	RT (min)	Plate count	RT (min)	Plate count
	0.9	2.105	2069	2.606	2176
	1.1	3.625	2912	4.419	2849
Changes in buffer ratio	65:35	2.263	2956	2.475	2252
	35:65	3.643	3409	4.468	3319

Ruggedness

The developed method is rugged by different analyst, different time intervals and the method did not significantly affect the recoveries, peak area and retention time of Lisinopril Dihydrate and Hydrochlorothiazide indicating that the proposed method is rugged. The ruggedness values of Lisinopril dihydrate and Hydrochlorothiazide are shown in Table 7.

Table 7: The ruggedness values of Lisinopril dihydrate and Hydrochlorothiazide

S.No	Lisinopril Dihydrate		Hydrochlorothiazide	
	RT(min)	Area	RT(min)	Area
1	2.361	793124	3.991	4301865
2	2.36	799158	3.99	4312874
3	2.364	791245	3.991	4286523
4	2.360	787654	3.99	4228579
5	2.361	790258	3.995	4301287
6	2.365	782215	3.99	4308526
Avg.	2.36183	790609	3.991	4289942.3
Stdev.	0.00214	5641.446	0.00194	31366.275
%RSD	**0.09**	**0.71**	**0.05**	**0.73**

CONCLUSION

The present investigation in the manuscript was a new analytical method development and its validation as per ICH guidelines. The chromatographic separation was achieved by using Waters e-2695 empower-2 HPLC instrument in Phenominex C-18 (250 mm ×4.6mm × 5 µ) column and the flow rate is 1.0 ml/min by using Potassium di hydrogen orthophosphate buffer as mobile phase. The both compounds were well separated and the retention time for Lisinopril Dihydrate and Hydrochlorothiazide is 2.347 min and 3.994 min respectively. The proposed method was validated and it was found to be simple, specific, precise and linear. Hence, the proposed method can be used for the routine analysis of both the compounds in pharmaceutical dosage form or API.

ACKNWOLEDGEMENT

The authors are thankful to Bio-Leo Labs, Hyderabad for providing standard, drug samples and facilities to carry out the work.

REFFERENCES

1. www.wikipedia.org/wiki/Lisinopril
2. www.wikipedia.org/wiki/Hydrochlorothiazide

3. Erdal Dinc, Dumitru Baleanu. Spectrophotometric quantitative determination of cilazapril and Hydrochlorothiazide in tablets by chemometric methods. J Pharm Biomed Anal 2002; 30(3): 715-23.
4. Paraskevas G, Atta-Politouhave J, Koupparis M. Spectrophotometric determination of lisinopril in tablets using 1-fluoro-2, 4-dinitrobenzene reagent. J Pharm Biomed Anal 2002; 29: 865-72.
5. El-Yazbi Fawzy A, Abdine Heba H, Rasha A. Shaalan. Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. J Pharm Biomed Anal 1999; 19: 819-27.
6. Basavaiah Kanakapura, Tharpa Kalsang, Salmara Ganeshbhat Hiriyannaand Basavaiah K Vinay. Spectrophotometric Determination of Lisinopril in Pharmaceuticals Using Ninhydrin - a Modified Approach. J Food Drug Anal 2009; 17(2): 93-99.
7. Alaa El-Gindy, Ahmed Ashour, Laila Abdel-Fattah, Marwan M Shabana. Spectrophotometric, spectrofluorimetric and LC determination of lisinopril. J Pharm Biomed Anal 2001; 25: 913-22.
8. Bankey S, Tapadiya G G, Saboo S S, Bindaiya S, Jain Deepti, Khadbadi S S. Simultaneous Determination of Ramipril, Hydrochlorothizide and Telmisartan by spectrophotometry. Int J Chem Tech Res 2009; 1(2): 183-8.
9. Esra S Aktas, Lale Erso, Olcay Sagırl. A new spectrofluorimetric method for the determination of lisinopril in tablets. Il Farmaco 2003; 58(2):165-8.
10. Nevin E, Murat K. Comparison of High-Performance Liquid Chromatography and Absorbance Ratio Methods for the Determination of Hydrochlorothiazide and Lisinopril in Pharmaceutical Formulations. Analytical letters 1999; 32(6):1131-41.
11. Meyyanathan SN, Rajan S, Muralidharan S, Arunadevi Birajdar S, Suresh B. A validated RP-HPLC method for simultaneous estimation of nebivolol and Hydrochlorothiazide in tablets. Indian J Pharm Sci 2008; 70(5): 687-9.
12. Erturk Sıdıka, Sevil Muge, Cetin, Sedef Atmaca. Simultaneous determination of moexipril hydrochloride and Hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid chromatographic methods. J Pharm Biomed Anal 2003; 33: 505-11.
13. Olcay Sagirli, Lale Ersoy. An HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection. J Chromatogr B 2004; 809:159-65.

14. Deepali D. Wagh, Pradip Dhore, Dipali S. Jain, D.R.Mundhada, Method Development And Validation Of Lisinopril And Hydrochlorothiazide In Comined Dosage Form By RP-HPLC, International Journal of Pharm Tech Research, 2012, 4(4):1570-1574.
15. Alaa El-Gindy, Ahmed Ashour, Laila Abdel-Fattah, Marwan M Shabana. Spectrophotometric and HPTLC-densitometric determination of lisinopril and Hydrochlorothiazide in binary mixtures. J Pharm Biomed Anal 2001; 25: 923-31.